

**Some Macroscopic and Microscopic Observation on The Pituitary Gland of Adult Male Donkey
(*equus-asinus*)**

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ABSTRACT

This research was performed using gross anatomy and a light microscope to detect some morphological and functional relationships of the pituitary gland in adult male donkey. For the anatomical and histological characteristics of the donkey pituitary, 14 adult normal animals were selected; all morphological descriptions were collected prior to preservation and midsagittal sectioning. Pituitary samples were preserved in 10% nutrient buffered formalin then it was processed and stained with routine H&E stain, Masson Trichrome and modified staining methods (AB+PAS - Orange G+PAS). With an average of 1.6 g, the weights ranged from 1.3g to 1.7g, with the diameter of the gland being (length 19.00 mm, width 20.7mm). The shape of the gland was round flattened; the anterior lobe was brown in color, but the posterior lobe was pale. Microscopically; a thin layer of C.T capsule derived from Dura matter was enclosed around the gland. There are two main components of the donkey pituitary: completely formed Adenohypophysis and neurohypophysis; pars distalis and pars intermedia are the Adenohypophysis. It was made up of the "adenohypophysis" epithelial glandular component and the "neurohypophysis" nervous component. The pars distalis was located laterally and pars nervosa in center of gland. Three major cell groups were composed of Pars distalis: acidophils, basophils and chromophobes. Most of the acidophils were Lateral of the gland and few cells in middle. The gonadotropes basophilis of donkey were located around the capillary sinusoids.

Keywords: *pituitary; morphology; donkey; Adenohypophysis; pars distalis*

INTRODUCTION

The pituitary gland has a major role in controlling important body functions; it is endocrine gland, it has been identified as master endocrine organ; its parenchymal cells of pituitary synthesize certain hormones which regulate specific tissue or organs of the body (William, 1993). These hormones that directly affect the activities of other endocrine glands in animal body, these hormones have also significant purpose in the regulation of body metabolic rate, growth and reproduction (Hussein, 2014; Martinez, 2011; Marieb, and Hoehn, 2012).

The pituitary gland comprises of three important parts (anterior, posterior and intermediate lobe), The anterior lobe (pars buccalis) was the major and largest lobe of the equine hypophysis, the anterior lobe of cattle, humans, rats, and rabbits, is greater than the posterior lobe (Herring, 1908). The intermediate lobe and the cleft Split the posterior lobe from the anterior lobe. The intermediate cleft divided the gland into two separate and different parts, the anterior lobe and the posterior lobe (Trautman, 1909a).

The pituitary lies in a minor shallow hypophyseal fossa "sella turcica:" a depression in the cavity of

basi sphenoid bone; it is covered with a thick fibrous sheet derived from dura mater through which the infundibulum stalk permits, communicating the gland with the hypothalamus (Trautman, 1909b). Dura mater covers the Major parts of gland except an opening through which the hypophyseal stalk passes (Dyce *et al.*, 1996).

Donkey is a member of the equidae. it is considered to be most important for their extreme environment adaptation. There is a distinct information about the normal anatomical and histological structure of the pituitary gland of the male donkey especially the cytology of Adenohypophysis (Francis and Mulligan, 1949).

The donkey has played an essential role in most regions of the country used as a means of transportation and play a remarkable role in agricultural progress (Hassan, 1995).

The pars distalis forms the largest portion in anterior lobe which is considered overall regulator of peripheral endocrine functions by synthesis and secretion of six major hormones (Page, 1994; Tucker, 1999; Greaves, 2007).

The adenohypophyseal cell types (acidophils, basophils and chromophobes) can be differentiated by using a combination of acidic and basic stains, due to the affinities of Specific secretory granules for acidic and basic dyes (Ross *et al.*, 1989). Acidophilic can be divided into GH producing cell and PRL producing cells (Kovacs and Hovarth, 1981). acidophiles Cells secreting GH and PRL stain with orange or light green, whereas basophils (Gonadotrophs and Adrenocorticotrophic cells) stains with basic dyes and some cells which stain poorly are termed as chromophobes. Depending on their affinity for various acidic and basic stains, two groups of cells in the horse's anterior lobe were known as chromophilic or chromophobic (Flesch, 1884). By using different staining mixtures, Pars distalis cells have been classified into acidophilic (Positive Orange G) and basophilic (positive PAS) and Chromophobes, the chromophobic cells were named chief cells because of their resemblance to cells in the peptic glands. (Trautman, 1909a).

The entire parenchyma is comprised of cell arrange as nests and cords within interconnecting network of small sinusoidal capillaries. Adenohypophyseal cells produce six hormonal cells as growth hormone (GH), prolactin (PRL), adrenocorticotrophic hormone (ACTH), follicle stimulating hormone (FSH), luteinizing hormone

(LH) and thyroid stimulating hormone (TSH) (Rahman *et al.*, 2011).

Several previous histological studies have been carried out in the mammalian pituitary in addition to the gross morphology; the main histological studies in equine and hypophysis have been termed by (Bailey, 1928; Rasmussen, 1934, 1938a; Trautman, 1909a and 1909b and Herring, 1908).

The aim of this research is to provide a gross morphological picture of the pituitary gland in adult male donkey for ongoing investigations for the relationship between environmental adaptation of the donkey and their special pituitary structure

MATERIAL AND METHODS

The donkey pituitary glands were dissected out in this study. Anatomical observations of their location, shape, diameter and weight were noticed. All these parameters were obtained from the pituitary of 14 apparently healthy adult males donkey obtained from local markets from Menoufia governorate shortly after slaughtering in anatomy department, the average age was 3-7 years old.

Technique of Removal and Trimming

The pituitary glands of donkey were dissected from ventral side of head region by using saw and hummer. The mandible was first removed after the styloid process of hyoid bone was broken, the basisphenoid bone was gently cutted and removed after that the pituitary gland photographed for its location and related structure. The dura lining was cut after that the gland removed from its position in the sella turcica with using scalpel and hemostatic forceps; they were trimmed and weighed immediately after removal, the gland was kept intact and weighted. The pituitary diameter (length and width) were measured by digital caliper after that it was rinsed with 9% normal saline and photos were taken by digital camera. Pituitary glands were bisected mid sagittal to distinguish its anterior and posterior lobes.

Histological technique

Pituitary Samples for light microscope were preserved in 10% neutral buffered formalin for 2-3 days, dehydrated in a serial dilution of alcohols, cleared in xylol and embedded in paraffin wax. Embedded tissues were cut and stained with Haematoxylin and eosin; other routine stain as Masson trichrome was used to demonstrate collagen fibers (Rasmussen, 1924). Special

combination stain such as PAS, Orange G method were used for differential staining of various cell types (acidophils and basophilis) and Alcian blue-periodic acid Schiff (PAS) for detection of acidic mucopolysaccharides (Pearse, 1968).

RESULT and DISCUSSION

The pituitary gland of donkey showed a great difference in morphological and histological structures from the other mammalian species.

Anatomical results

The present study found that the pituitary gland in donkey was unpaired organ. It was Located at the base of the brain, the pituitary gland of donkey weighting approximately 1.5gm and about 17 m in length, 20 m in width. The pituitary gland weight of the donkey was similar to the weight of camel pituitary (Rahman *et al.*, 2011).

The pituitary gland consisted of two parts (anterior and posterior) which can be identify by naked eye which different from result of (Hussein and Mahmoud, 2014). It was covered mainly the inter peduncular fossa and suspended at the base of the brain by a narrow infundibular stalk. The gland was situated in sella turcica of basisphenoid bone Fig1(A.B), This is agreement with (William, 1993; Brams, 1932; Brien, 1996; Pantic, 1975) in equine, ruminant, carnivore and pig.

It was flattened ovoid in form with the same peanut size, the anterior lobe has Brown in color while the posterior lobe was pale this shape similar to pituitary of carnivore but it was different from other species; it was thick and elongated in sheep and it was rounded with convex ventral surface in pig This obtained results in agreement with previous result of (Das, 1971). Fig 2(A.B).

In this study we noticed that the anterior lobe was found to be distinctly larger than posterior lobes, which confirmed previously published data by Cowan, *et al.*, (2008). Trautman, (1909a;1909b) and Herring, (1908) in cattle, human and rabbit.

It was surrounded by very thin firmly attached capsule which is part from the dura mater called the (sella diaphragm). This capsule enclosed the pituitary gland and tightly attached with it except at the upper part of the pituitary gland infundibulum stalk as described for all domestic mammals (Dyce et al (1996).

The current observations revealed that the gland of donkey could be divided into two parts, the outer Adenohypophysis and the inner

neurohypophysis. The obtained results as described in domestic mammals by Dellmann, (1993); Banks, (1993); Bacha, (2000). The Adenohypophysis was subdivided into a proximal part was called pars tuberalis around stalk, the rostro lateral part called pars distalis and the caudodorsal part called pars intermedia which closely attached to the pars nervosa without clear cleft. Fig (3). Anterior and posterior pituitaries defined as two separate parts of different embryology, anatomy, and structure in Horse, ruminant, carnivores and pig This is in accordance with (Chakravarthy, and Mariappa, (1975). Copenhaver, et al. (1978). Jubb, and. McEntee, (1955).

The present result from the mid sagittal section of the donkey pituitary is bilaterally symmetrical. The donkey pituitary gland could be divided into three parts: pars nervosa, pars distalis and pars intermedia Fig3B. The hypophyseal cleft was not obvious between pars distalis and pars intermedia in donkey pituitary Fig3A. This result agrees with a report of Trautman, (1909a). The pars intermedia is a narrow region between the pars distalis and pars nervosa, The Neurohypophysis separated into three regions, median eminence, the infundibular stalk and a thin layer of glandular tissue of pars tuberalis Fig 1B.

Histological results:

The current results showed that the donkey pituitary gland has no true special capsule, it was clear lobulated gland. The gland was covered by a thinner layer of capsule with fine connective tissue, which consisted of mainly collagen fibers and fine reticular fiber. The parenchyma of donkey pituitary had two the outer adenohypophysis and the inner neurohypophysis. These findings resemble the previous result in bovine pituitary of Jubb, and McEntee (1955).

Adenohypophysis.

The cells of donkey adenohypophysis arranged in cell cords or clusters, and formed the following parts: the pars distalis and the pars intermedia Fig 4.

Pars distalis

The pars distalis of donkey pituitary was the largest portion of the whole gland; They were surrounded by a fine connective tissue Stroma, which contained large sinusoidal capillaries, the cells of the pars distalis were divided into three main types: acidophils, basophils and chromophobes Fig 4(a,b). The cytoplasmic of the

acidophil cells were red, and the nuclei were deep blue. The cytoplasmic of the basophil cells were light blue, and the nuclei were deep blue. Moreover, the chromophobe cells were negative staining by HE, the cytoplasmic were light blue or colorless, and the nuclei were light blue. The chromophobe cells were rounded or polygonal-shaped, the anterior lobe of donkey pituitary contained an area in the central part in which the chromophobes are found Fig5, the present study agrees with Nakane, (1970).

Acidophil cell (AC).

The majority of cells were situated in the lateral and central parts of the pars distalis, the few population were in the interior part. The acidophil cells were round to ovoid in shape and medium-sized. The nuclei were rounded and peripherally located. The acidophils were smaller than basophils Fig 6(A).

Somatotrophic cell (SC). The somatotrophic cells arranged in clusters along the capillary. They were rounded or polyhedral, the nuclei were round and located centrally. The cytoplasmic of somatotrophic cells could be stained orange by orange G Fig6 B.

Lactotrophic cell (LC). The lactotrophic cells arranged singularly, and randomly distributed within the pars distalis. Fig6 B.

Basophil cell (BC). The basophil cells of donkey pituitary were slightly larger than the acidophil

cells; they mainly concentrated around blood vessels and center of gland.

Gonadotrophic cell (GC). The gonadotrophic cells were around the capillary sinusoids as differed from other mammalian species while the cells distributed throughout widely the pars distalis (Herring, P. T. (1908). Fig 6(A). there were more cell types than acidophils, basophils, and chromophobes on the basis of the size and shape of their secretory granules as thyrotrophs cells, gonadotrophs cells and corticotrophs cells (Barnes., 1962; Nakane, 1970; Phifer and Orth, 1970 and Brown, 1971).The donkey pituitary Chromophobes cells are fewer than chromophilis and appeared as classis of small cells. They show little affinity for dyes in histological section. These cells reach the size of acidophils and basophils. They were devoid of secretory granules. (Barnes, 1962 and Nakane, 1970). they were considered as stem cell which could differentiated to acidophilis and basophilis cell (Fig5.) The distinct area in this gland is the pars intermedia. The present study emphasized that the donkey pituitary gland has a distinct pars intermedia. It lied closely to the neurohypophysis (Fig5); Pars intermedia consist of chromophobes and basophil cells basophiles arranged in follicles like structure around blood sinusoids Fig7A. pars nervosa showing unmyelinated nerve fibers pituicytes (green arrow) and blood capillaries Fig7B.

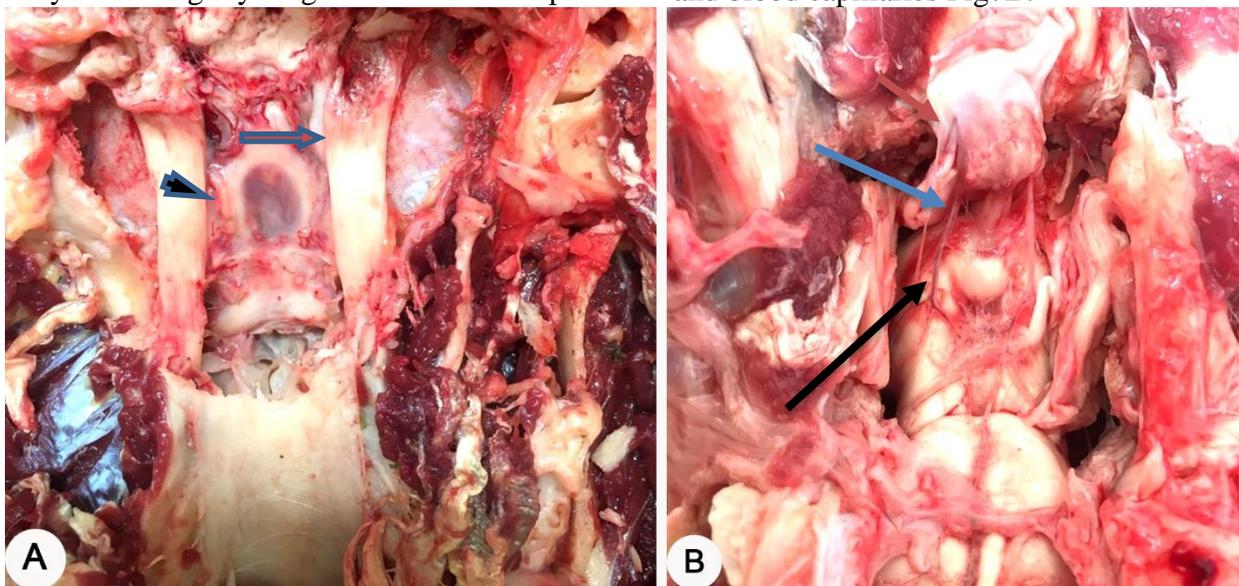


Fig. 1. adult donkey pituitary gland in its normal location inside the basisphenoid fossa from the ventral surface, **A** “black arrow refer to pituitary gland surrounded by diaphragma Sella of dura mater, the orange arrow refer to auditory tube .**B**” Orange arrow referred to pituitary gland after removal from the fossa with intact infundibular stalk (blue arrow), a black arrow (mammillary body).

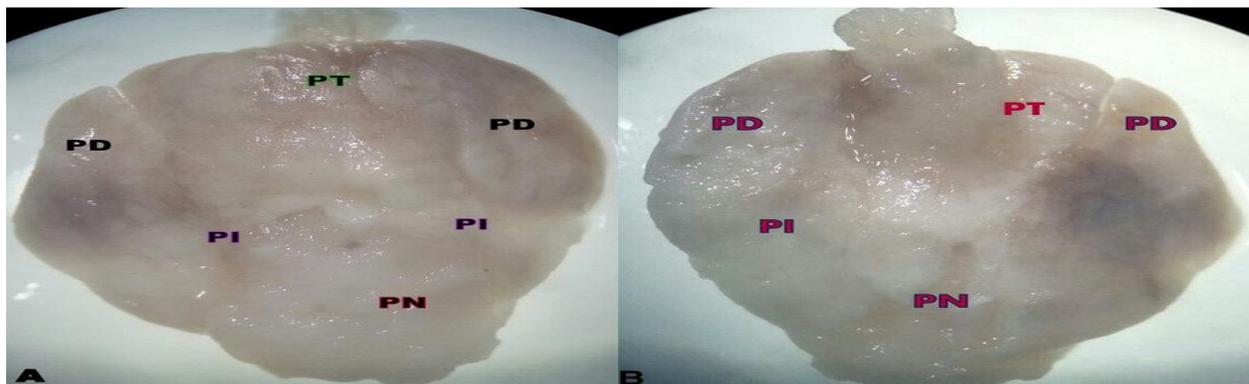


Fig. 2. Adult pituitary gland of donkey with stereo microscope. A) photograph of dorsal surface of the donkey pituitary gland. B) Photograph of Ventral surface of the donkey pituitary gland.

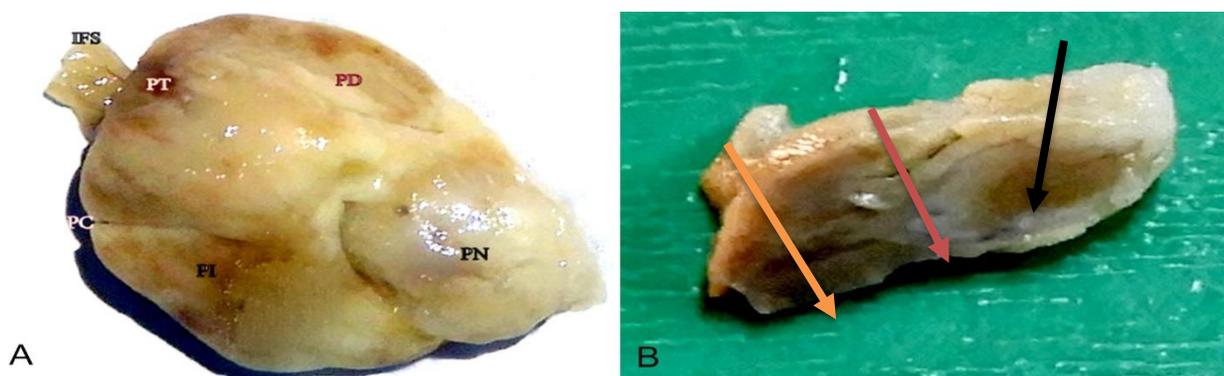


Fig. 3. Adult donkey pituitary gland photographed by digital camera showing the different region of anterior and posterior lobe. (A) IFS (infundibular stalk), PT (parstuberalis), PD (Parsdistalis), PI (pars intermedia) and pc (shallow pituitary cleft). (B) The midsagittal section of donkey pituitary gland showing the pars distalis completely surround the pars nervosa, the green AND black arrow (PD), orange (PN)

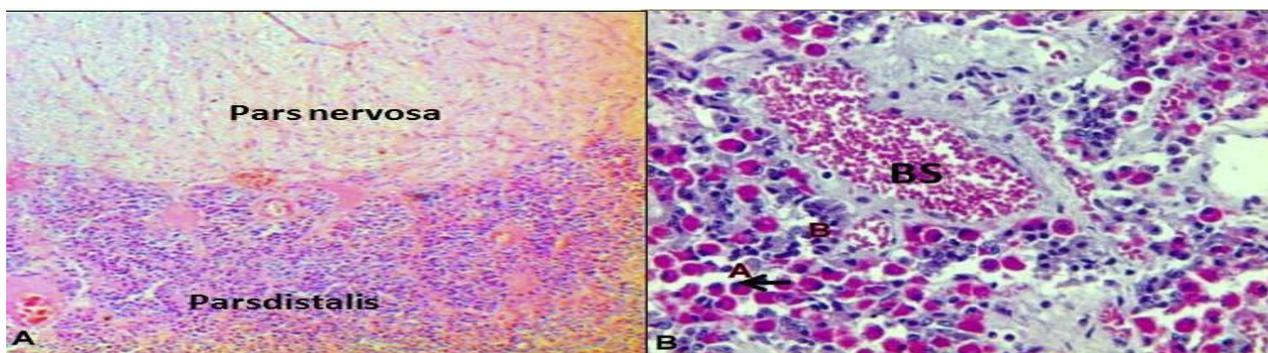


Fig. 4. (A) Alight photomicrograph showed the two different structure of the donkey pituitary gland lobes. (B) A photomicrograph showed (A), acidophilic; (B), Basophilic; Most Basophiles arranged around blood sinusoids

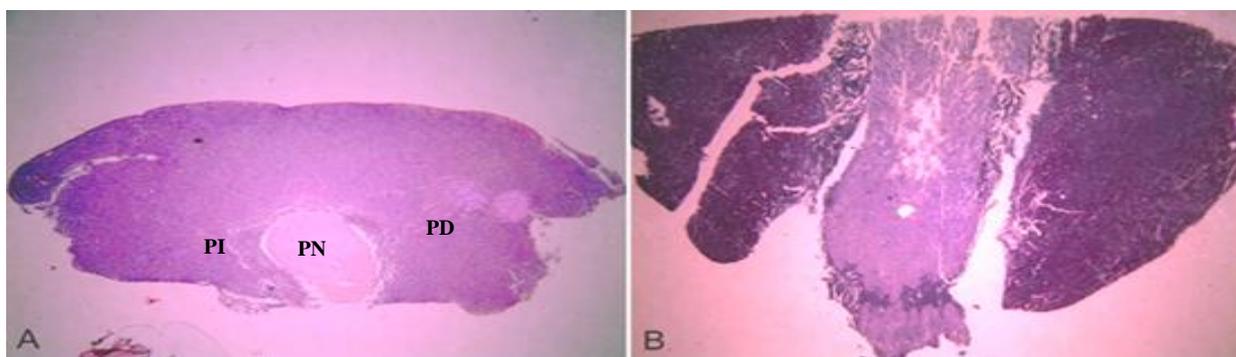


Fig. 5. A) light photomicrograph of pituitary showing Parsdistalis (PD), pars nervosa (PN) and pars intermedia (PI) (x40). B) the Parsdistalis surround the pars nervosa.

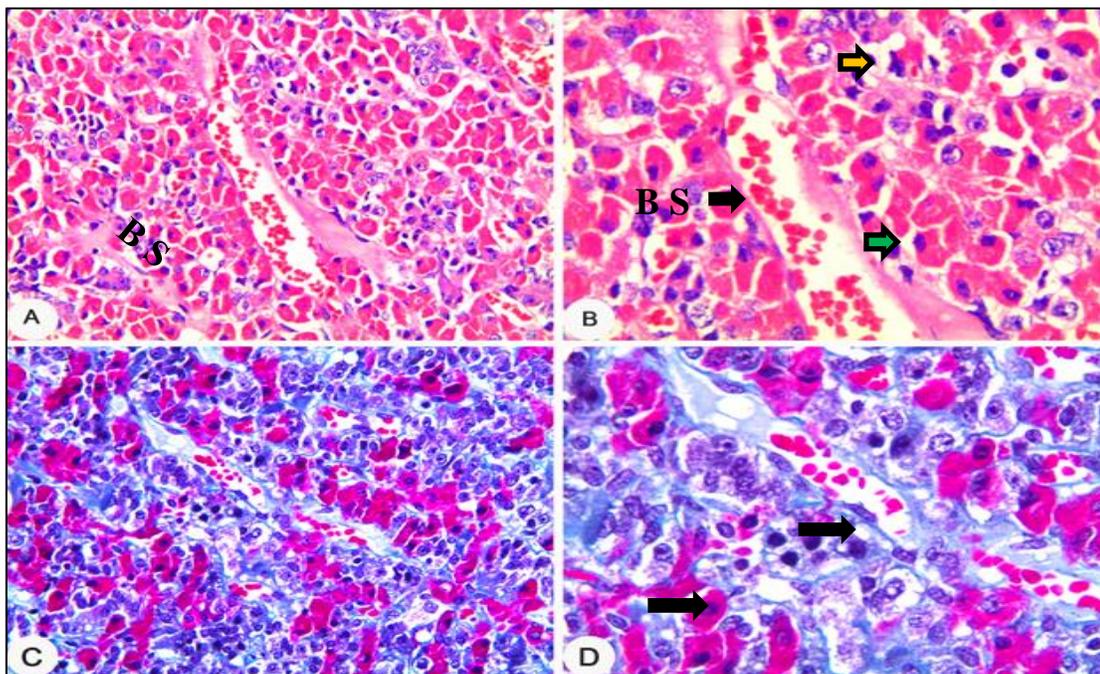


Fig. 6. A light photomicrograph of pars distalis showing: B; blood sinusoid (B S), acidophiles (black arrow), basophiles (green arrow) and chromophobes (yellow arrow) (H&E); C, D: collagen fibers around blood sinusoids and cell cords (arrow) (Masson trichrome) .

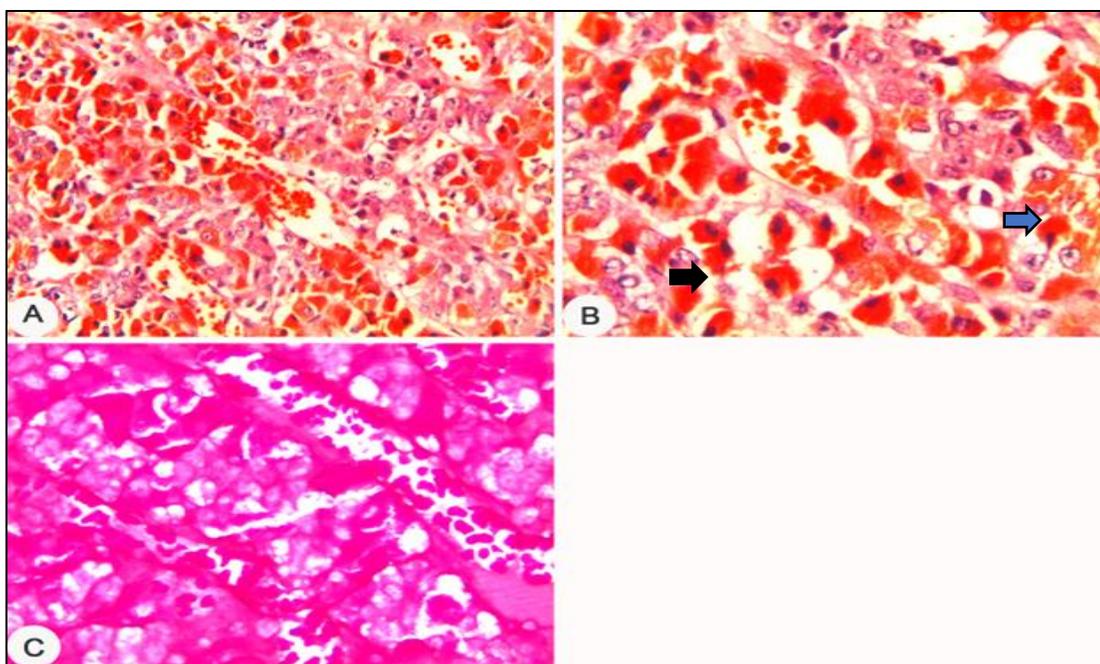


Fig. 7. Light photomicrograph of pars distalis cell were stained with (OG-PAS) technique, the cells were stained as acidophils (orangeophils). B: somatotropes (blue arrow) and lactotropes (black arrow); C: basophiles with positive PAS reaction (PAS-OG stain).

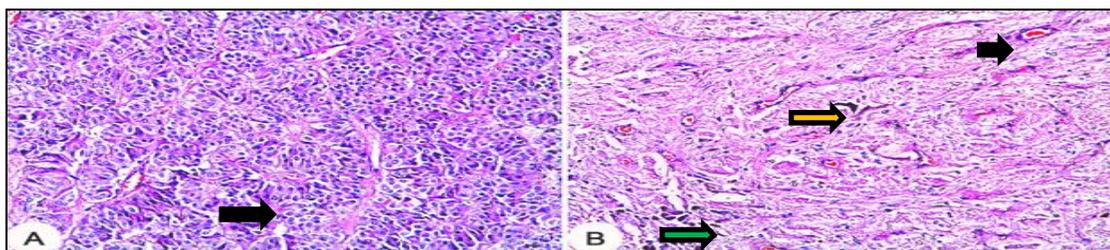


Fig. 8. light photomicrograph of A: The present study revealed that the donkey has well-developed pars intermedia. It lied closely to the neurohypophysis

CONCLUSION

In this research on the histo anatomical structures of the donkey pituitary gland data obtained from 14 adult donkey males been studied. The histomorphological features of donkey pituitary show a great difference from other mammalian species, The pars nervosa locate in the centre of the gland, and surrounded by the pars intermedia and the pars distalis. No intermediate cleft is present and gonadotrophs basophils mainly locate around blood vessels.

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